Biochem. J. (1980) **186**, 343–350
Printed in Great Britain

Purine Metabolism in Mesophyll Protoplasts of Tobacco (*Nicotiana tabacum*) Leaves

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(*Received* 9 *August* 1979)

The overall metabolism of purines was studied in tobacco (*Nicotiana tabacum*) mesophyll protoplasts. Metabolic pathways were studied by measuring the conversion of radioactive adenine, adenosine, hypoxanthine and guanine into purine ribonucleotides, ribonucleosides, bases and nucleic acid constituents. Adenine was extensively deaminated to hypoxanthine, whereupon it was also converted into AMP and incorporated into nucleic acids. Adenosine was mainly hydrolysed to adenine. Inosinate formed from hypoxanthine was converted into AMP and GMP, which were then catabolized to adenine and guanosine respectively. Guanine was mainly deaminated to xanthine and also incorporated into nucleic acids via GTP. Increased RNA synthesis in the protoplasts resulted in enhanced incorporation of adenine and guanine, but not of hypoxanthine and adenosine, into the nucleic acid fraction. The overall pattern of purine-nucleotide metabolic pathways in protoplasts of tobacco leaf mesophyll is proposed.

Tissues of many plant species have been found to contain purine bases, nucleosides and nucleotides, as well as several enzymes taking part in purine metabolism (Brown, 1975). However, not much is known about the relative rates of the alternative pathways of purine metabolism in plants (Suzuki & Takahashi, 1977).

The aim of the present investigation was to study the metabolism of purine compounds in the isolated plant cells with radioactive adenine, adenosine, hypoxanthine, and guanine. Isolated mesophyll protoplasts seemed to be especially appropriate for such studies, because they are highly organized and differentiated, with a well-defined assimilation function; furthermore they form a uniform suspension in liquid medium. Additionally, the cultivation of protoplasts enables studies of purine metabolism under conditions of more intense RNA synthesis.

This is the first study of overall purine metabolism in isolated protoplasts.

Experimental

Reagents

Chemicals were purchased from the following sources: purine ribonucleotides, ribonucleosides, bases, 5-phosphoribosyl 1-pyrophosphate, ribose 1-phosphate, NAD^+ and phenyl phosphate (Calbiochem, Los Angeles, CA, U.S.A.); Tris, PPO (2,5-diphenyloxazole) (Serva, Heidelberg, Germany); POPOP [1,4-bis-(5-phenyloxazol-2-yl)-benzene] (Fluka A.G., Buchs, Switzerland); ethoxyethanol (UCB, Bruxelles, Belgium); 8-^{14}C-labelled purine compounds (adenine, adenosine, hypoxanthine, inosine, guanine, guanosine) (The Radiochemical Centre, Amersham, Bucks., U.K.); other reagents (Polskie Odczynniki Chemiczne, Gliwice, Poland). Cellulase 'Onozuka' R-10 and Macerozyme R-10 were purchased from Kinki Yakutt Co., Nishinomiye, Japan.

Isolation and cultivation of protoplasts

Protoplasts of tobacco (*Nicotiana tabacum* cv. *Xanthi*) mesophyll cells were isolated from fully developed leaves of 60–80-day-old plants grown in a greenhouse. Protoplasts were prepared in a sterile box by the one-step method of Power *et al.* (1971), by using 2% (w/v) cellulase R-10 and 0.5% (w/v) Macerozyme R-10. Briefly, after 2h of preplasmolysis of leaf tissue fragments in a modified cell-protoplast-washing medium (CPW medium) (Cocking & Peberdy, 1974) containing (per litre) 27.2mg of KH_2PO_4, 101mg of KNO_3, 1480mg of $CaCl_2,2H_2O$, 246mg of $MgSO_4,7H_2O$, 0.16mg of KI, 0.025mg of $CuSO_4,5H_2O$, 36.4g of mannitol and 36.4g of sorbitol, both enzymes were added and the mixture was incubated at room temperature for 8h. Protoplasts released from the tissue were passed through a 80μm-mesh nylon screen, and washed five

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times with CPW medium and once with K_3 medium (Kao *et al.*, 1974), each washing being followed by centrifugation at 100*g* for 3 min; the protoplasts so obtained were used for experiments or for cultivation. Cultivation was carried out in darkness at 26°C in K_3 medium in 10 cm-diameter Petri dishes (10^5 cells/ml).

Incubation of protoplasts with radioactive purines

In these experiments a 2.5% (v/v) suspension of newly isolated or cultivated protoplasts in K_3 medium was used. The protoplast suspension (80 μl) was incubated with 10 μl of a 2 mM (40 mCi/mmol) solution of 8-^{14}C-labelled precursor (adenine, adenosine, hypoxanthine or guanine) and 10 μl of K_3 medium in air atmosphere for 60 min at 30°C with shaking by hand at 5 min intervals.

Separation and determination of radioactive purine compounds in protoplasts

The extraction of labelled metabolites, separation of purine ribonucleotides, ribonucleosides and bases, as well as radioactivity measurements, were performed as described by Henderson *et al.* (1974). The total acid-insoluble radioactivity, equivalent to nucleic acids synthesized, was measured as described by Snyder & Henderson (1973). For separation and determination of radioactive purine compounds, 10 μl of incubation mixture (total volume 0.1 ml) was used. The radioactivity of individual purine compounds was expressed as c.p.m./total incubation mixture after 60 min incubation. Concentration of individual radioactive purine compounds was calculated from the radioactivity of these compounds and the radioactivity of the precursor corresponding to 20 nmol of each purine precursor used in experiments. The concentration of radioactive purine compounds in protoplasts was expressed in nmol/ml of packed cells after 60 min incubation.

Assays of enzymic activities in protoplast extracts

The suspension (25%, v/v) of newly isolated protoplasts in 50 mM-Tris/HCl buffer, pH 8.0, was homogenized in a Potter glass homogenizer in an ice/water bath and centrifuged at 20000*g* and 4°C for 60 min ($r_{av.}$ 10 cm); the supernatant was used for enzyme-activity detection.

The incubation mixture (0.1 ml) contained 50 mM-Tris/HCl buffer, pH 8.0, 0.04 mg of protein and the following.

(*a*) For adenine phosphoribosyltransferase (EC 2.4.2.7), hypoxanthine phosphoribosyltransferase (EC 2.4.2.8) and guanine phosphoribosyltransferase (EC 2.4.2.8) activities: 0.1 mM-[8-^{14}C]adenine, -[8-^{14}C]hypoxanthine and -[8-^{14}C]guanine respectively, 1.5 mM-5-phosphoribosyl 1-pyrophosphate and 5 mM-$MgCl_2$.

(*b*) For purine nucleoside phosphorylase (EC 2.4.2.1 and EC 2.4.2.15) activities: 1.5 mM-ribose 1-phosphate and 0.1 mM-purine base ([8-^{14}C]adenine, [8-^{14}C]hypoxanthine or [8-^{14}C]guanine) for nucleoside synthesis, or 0.1 mM-nucleoside ([8-^{14}C]adenosine or [8-^{14}C]inosine) and 20 mM-KH_2PO_4 for nucleoside degradation.

(*c*) For adenosine kinase (EC 2.7.1.20) and inosine kinase (EC 2.7.1.73) activities: 10 mM-ATP, 5 mM-$MgCl_2$ and 0.1 mM-[8-^{14}C]adenosine and -[8-^{14}C]inosine respectively.

(*d*) For nucleoside phosphotransferase (EC 2.7.1.77) activities: 10 mM-phenyl phosphate or 10 mM-ATP, 5 mM-$MgCl_2$ and 0.1 mM-[8-^{14}C]adenosine or -[8-^{14}C]inosine.

(*e*) For AMP aminohydrolase (EC 3.5.4.6), adenosine aminohydrolase (EC 3.5.4.4), adenine aminohydrolase (EC 3.5.4.2) and guanine aminohydrolase (EC 3.5.4.3) activities: 0.1 mM-[8-^{14}C]AMP, -[8-^{14}C]adenosine, -[8-^{14}C]adenine or -[8-^{14}C]guanine respectively.

(*f*) For adenosine nucleosidase (EC 3.2.2.1) and inosine nucleosidase (EC 3.2.2.2) activities: 0.1 mM-[8-^{14}C]adenosine and -[8-^{14}C]inosine respectively.

(*g*) For xanthine oxidoreductase (EC 1.2.3.2) activity: 0.1 mM-[8-^{14}C]hypoxanthine and 0.4 mM-NAD^+.

(*h*) For ribonucleotide-dephosphorylating activity (EC 3.1.3.1, EC 3.1.3.2 and EC 3.1.3.5): 0.1 mM-[8-^{14}C]AMP.

The specific radioactivity of all labelled compounds used in experiments was 5 mCi/mmol.

All reactions were carried out for 10 min at 30°C and stopped by an addition of 5 μl of cold 4.2 M-$HClO_4$. After 10 min the samples were neutralized with 4.45 M-KOH and centrifuged at 2000*g* and 2°C for 5 min. Then 0.1 ml of each supernatant was spotted on Whatman 3MM chromatograms together with appropriate carriers and the chromatograms were developed by ascending chromatography in aq. 5% (w/v) Na_2HPO_4 (Barankiewicz & Henderson, 1977). For guanine deaminase the chromatograms were developed in butan-1-ol/0.1 M-HCl/ethoxyethanol (4:1:1, by vol.; Farkas & Singh, 1975), and for xanthine oxidoreductase, in propan-1-ol/water (3:2, v/v; Barankiewicz & Jeżewska, 1972). Spots were localized under u.v. light and their radioactivity was counted in a Packard Tri-Carb liquid-scintillation spectrometer with toluene containing 0.01% (w/v) POPOP and 0.5% (w/v) PPO as scintillator. Protein was determined as described by Lowry *et al.* (1951).

Enzyme activities were expressed in nmol of product/60 min per mg of protein.

All results presented are averages of triplicate determinations in three experiments; average deviation of individual results from the mean was less than 9%.

Results

After incubation of newly isolated protoplasts with [8-^{14}C]adenine, most radioactivity was found in adenosine, hypoxanthine, free adenine nucleotides, the nucleic acid fraction and inosine (Table 1). In comparison the cultured protoplasts showed the highest radioactivities in the nucleic acids fraction and hypoxanthine (7- and 4-fold increases respectively). Radioactivity found in xanthosine increased 4-fold, and that of xanthine and guanine 2-fold, whereas that in adenine nucleotides, adenosine and IMP was about half as much.

After incubation of newly isolated protoplasts with [8-^{14}C]adenosine, most radioactivity was found in adenine (Table 2). Inosine and the nucleic acid

Table 1. *Adenine metabolism in tobacco leaf protoplasts*

Protoplasts were isolated and cultivated in K_3 medium as described in the Experimental section. Protoplast suspension (2%, v/v) in K_3 medium containing 0.2mM-[8-^{14}C]adenine (40mCi/mmol; final incubation volume 0.1ml) was incubated for 60min at 30°C. Measurements of radioactivity of individual purine compounds and calculations of their concentrations were carried out as described in the Experimental section.

	Radioactivity			
Protoplasts ...	Newly isolated		Cultured	
Purine compound	c.p.m./ incubation mixture	nmol/ml of packed cells	c.p.m./ incubation mixture	nmol/ml of packed cells
Purines in nucleic acids	7240	115	52400	828
ATP + ADP + AMP	10600	169	4160	66
Adenosine	14200	226	8210	130
GTP + GDP + GMP	1510	24	1360	21
Guanosine	1850	29	1630	26
Guanine	680	11	1260	20
IMP	750	12	450	7
Inosine	3270	52	3350	53
Hypoxanthine	11190	178	47310	747
XMP	690	11	380	6
Xanthosine	490	8	1780	28
Xanthine	480	8	1000	16
Uric acid	1890	30	830	13

Table 2. *Adenosine metabolism in tobacco leaf protoplasts*

Protoplast suspension (2%, v/v) was incubated with 0.2mM-[8-^{14}C]adenosine. For details, see Table 1.

	Radioactivity			
Protoplasts ...	Newly isolated		Cultured	
Purine compound	c.p.m./ incubation mixture	nmol/ml of packed cells	c.p.m./ incubation mixture	nmol/ml of packed cells
Purines in nucleic acids	10080	140	12120	165
ATP + ADP + AMP	5540	77	13230	180
Adenine	104110	1446	69890	951
GTP + GDP + GMP	6260	87	11020	150
Guanosine	2800	39	2710	37
Guanine	1260	17	5140	70
IMP	2010	28	4180	57
Inosine	15520	216	12640	172
Hypoxanthine	4100	57	4630	63
XMP	2160	30	3380	46
Xanthosine	1150	16	2790	38
Xanthine	1730	24	2060	28
Uric acid	1220	17	5440	74

fraction also showed relatively high radioactivities, and nucleotides of guanine and adenine were also more intensely labelled than other purine compounds. The cultured protoplasts showed about twice as much radioactivity in nucleotides of adenine and guanine, IMP and xanthine, and 4 times as much radioactivity in uric acid, as in newly isolated protoplasts. However, the incorporation of adenosine into nucleic acids was not enhanced, and degradation of adenosine to adenine was slower.

After incorporation of [8-^{14}C]hypoxanthine by newly isolated protoplasts, most radioactivity was found in adenine, guanosine and inosine (Table 3). Relatively low radioactivity was observed in the nucleic acid fraction. In comparison, the cultivated protoplasts showed 1.5–2 times as much radioactivity in inosine, the nucleic acid fraction, adenine nucleotides and xanthine.

After incubation of newly isolated protoplasts with [8-^{14}C]guanine, most radioactivity was found

Table 3. *Hypoxanthine metabolism in tobacco leaf protoplasts*
Protoplast suspension (2%, v/v) was incubated with 0.2 mM-[8-^{14}C]hypoxanthine. For details, see Table 1.

	Radioactivity			
Protoplasts ...	Newly isolated		Cultured	
Purine compound	c.p.m./ incubation mixture	nmol/ml of packed cells	c.p.m./ incubation mixture	nmol/ml of packed cells
Purines in nucleic acids	3870	46	6330	82
ATP + ADP + AMP	1480	18	2140	28
Adenosine	2540	30	1260	16
Adenine	31580	378	26470	341
GTP + GDP + GMP	1720	21	2280	29
Guanosine	17900	214	6000	77
Guanine	2160	26	790	10
IMP	640	8	700	9
Inosine	7030	84	12560	162
XMP	710	9	920	12
Xanthosine	2060	25	1510	19
Xanthine	690	8	1320	17
Uric acid	490	6	740	10

Table 4. *Guanine metabolism in tobacco leaf protoplasts*
Protoplast suspension (2%, v/v) was incubated with [8-^{14}C]guanine. For details, see Table 1.

	Radioactivity			
Protoplasts ...	Newly isolated		Cultured	
Purine compound	c.p.m./ incubation mixture	nmol/ml of packed cells	c.p.m./ incubation mixture	nmol/ml of packed cells
Purines in nucleic acids	25210	368	44580	627
ATP + ADP + AMP	8500	124	8530	120
Adenosine	1990	29	1490	21
Adenine	2260	33	2490	35
GTP + GDP + GMP	10820	158	4760	67
Guanosine	4590	67	1850	26
IMP	5690	83	2630	37
Inosine	2470	36	1850	26
Hypoxanthine	2880	42	2770	39
XMP	3770	55	2840	40
Xanthosine	2190	32	2420	34
Xanthine	122300	1785	201580	2835
Uric acid	3970	58	3200	45

Table 5. *Activities of purine-metabolism enzymes in extracts of tobacco leaf protoplasts*
Conditions of enzyme assays are described in the Experimental section.

Enzyme	EC no.	Enzymic activity (nmol of product/h per mg of protein)
Adenine phosphoribosyltransferase	2.4.2.7	165.5
Nucleotide-deposphorylating enzymes	3.1.3.1 / 3.1.3.2 / 3.1.3.5	933.8
Adenosine kinase	2.7.1.20	0.0
AMP aminohydrolase	3.5.4.6	0.0
Adenosine aminohydrolase	3.5.4.4	0.0
Adenine aminohydrolase	3.5.4.2	65.0
Adenosine phosphorylase	2.4.2.1	0.0
Adenosine nucleosidase	3.2.2.1	64.0
Hypoxanthine phosphoribosyltransferase	2.4.2.8	37.0
Inosine kinase	2.7.1.73	38.0
Inosine phosphotransferase	2.7.1.77	
with AMP		80.3
with phenyl phosphate		19.4
Inosine phosphorylase	2.4.2.1	0.0
Inosine nucleosidase	3.2.2.2	0.0
Xanthine oxidoreductase	1.2.3.2	4.6
Guanine phosphoribosyltransferase	2.4.2.8	36.5
Guanosine phosphorylase	2.4.2.15	0.0
Guanosine nucleosidase		0.0
Guanine aminohydrolase	3.5.4.3	73.9

in xanthine, the nucleic acid fraction, and nucleotides of guanine and adenine (Table 4). The cultured protoplasts showed 1.5 times as much radioactivity in the nucleic acid fraction and xanthine, but a 2-fold decrease of radioactivity in guanine nucleotides and guanosine, compared with newly isolated protoplasts.

Activities of the purine-metabolism enzymes, assayed in extracts of newly isolated protoplasts, are presented in Table 5. The AMP-dephosphorylating activity was the highest. Protoplast extracts exhibited phosphoribosyltransferase activities towards adenine, hypoxanthine and guanine, that towards adenine being 5 times as much as that for the remaining two purines. Anabolic and catabolic activities of purine nucleoside phosphorylase towards all purines and nucleosides used as substrate were absent; instead nucleosidase activity towards adenosine, but not towards inosine or guanosine, was found. Extracts exhibited both adenine aminohydrolase and guanine aminohydrolase activities, but no activities of AMP and adenosine aminohydrolases were detected. Also no adenosine kinase was found, whereas inosine kinase and inosine phosphotransferase activities were present; the latter were higher with AMP than with phenyl phosphate as substrate. Xanthine oxidoreductase activity was detectable, but it was very low.

Discussion

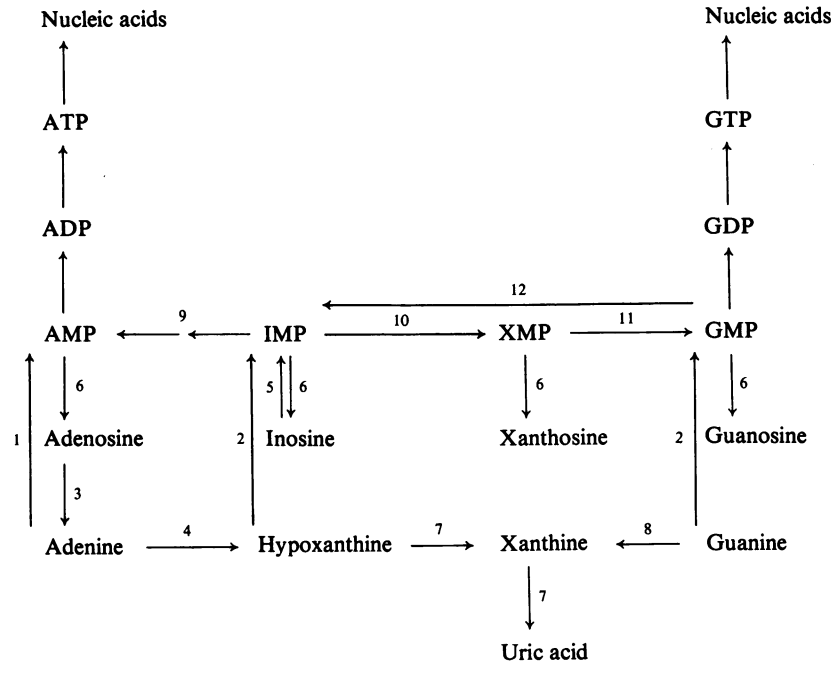
Results presented in Table 1 show that [8-^{14}C]adenine is metabolized in tobacco leaf protoplasts in two ways: it can be transformed into AMP and deaminated to hypoxanthine. Conversion into AMP is a one-step reaction catalysed by adenine phosphoribosyltransferase, an enzyme found in tobacco protoplasts (Table 5); this enzyme has been found in other plant species namely wheat (*Triticum vulgare*) (Price & Murray, 1969), barley (*Hordeum vulgare*) (Nicholls & Murray, 1968), potato (*Solanum tuberosum*) (Clark, 1974) and soya bean (*Glycine max*) (Anderson, 1977). Conversion of adenine into adenosine and further into AMP seems to be excluded in protoplasts, because of the lack of both purine nucleoside phosphorylase and adenosine kinase activities (Table 5). AMP synthesized from adenine is then phosphorylated to ADP and ATP, and is finally incorporated into nucleic acids. On the other hand, AMP may be dephosphorylated to adenosine by 5′-nucleotidase and/or non-specific phosphatases (Table 5, nucleotide-dephosphorylating enzymes). It seems that AMP is not deaminated to IMP, because AMP aminohydrolase was not detectable in protoplast extracts. However, the activity of this enzyme has been described for pea (*Pisum sativum*) seeds (Turner & Turner, 1961).

Accumulation of adenosine observed during the incubation of protoplasts with [8-^{14}C]adenine (Table 1) seems to result from the lack of adenosine aminohydrolase and adenosine kinase activities as well as from the inhibition of adenosine nucleosidase (an enzyme not found in animal tissue; Henderson & Patterson, 1973) by the high concentration of adenine. On the other hand, the presence of adenosine nucleosidase in protoplasts (Table 5) and the accumulation of radioactive adenine during protoplast incubation with [8-^{14}C]adenosine (Table 2) indicate that, in protoplasts, adenosine is hydrolytically degraded. Adenosine nucleosidase has been found also in barley leaves (Guranowski & Schneider, 1977), and this enzyme seems to play an important role in adenosine catabolism in plants. Adenine formed from adenosine or administered to protoplasts may be converted into adenylate by adenine phosphoribosyltransferase or deaminated to hypoxanthine by adenine aminohydrolase. The latter enzyme, found in several plant species (Barnes, 1959; Schlee & Reinbothe, 1965), seems to play an important role in tobacco leaf protoplasts, as the only enzyme taking part in deamination of the pool of adenine compounds. Conversely, deamination of adenine is a rather unimportant reaction in animal tissues (Henderson & Patterson, 1973). Labelled inosine found in tobacco leaf protoplasts incubated with [8-^{14}C]adenine or [8-^{14}C]adenosine (Tables 1 and 2) seems to originate from the following sequence of reactions: adenosine → adenine → hypoxanthine → IMP → inosine. Other routes potentially available for inosine formation seem to be excluded, because of the absence of adenosine aminohydrolase, AMP aminohydrolase and purine nucleoside phosphorylase in protoplasts (Table 5). Thus the incorporation of the label of adenosine and adenine (Tables 1 and 2) into xanthine and guanine compounds must follow the pathway adenosine → adenine → hypoxanthine → IMP → XMP → GMP.

In tobacco leaf protoplasts, hypoxanthine can be transformed into IMP by hypoxanthine phosphoribosyltransferase (Table 5), an enzyme also found in germinating wheat embryos (Price & Murray, 1969), but not in potato leaf extract (Clark, 1974). In contrast with animal tissues (Henderson & Patterson, 1973), the activity of hypoxanthine/guanine phosphoribosyltransferase in tobacco-leaf-protoplast extracts was lower than the adenine phosphoribosyltransferase activity. However, in isolated protoplasts the reutilization of hypoxanthine was greater than that of adenine (Tables 1 and 3). IMP formed can subsequently be converted into AMP (by adenylosuccinate synthetase, EC 6.3.4.4, and adenylosuccinate lyase, EC 4.3.2.2) and/or GMP via XMP (by IMP dehydrogenase, EC 1.2.1.14, and GMP synthetase, EC 6.3.4.1). Interconversion to AMP is about twice as much as that to GMP. AMP and GMP are, after further phosphorylation, incorporated into nucleic acids, but to a small extent (Table 3). Most of the purine nucleotides formed are catabolized by dephosphorylating enzymes (Table 5) to the respective nucleosides (Table 3). Adenosine is further degraded, as described above, to adenine, which accumulates; the lack of purine nucleoside phosphorylase and nucleosidases specific towards other nucleosides (Table 5) results in accumulation of guanosine, xanthosine and inosine. The latter nucleoside can be converted into IMP by inosine phosphotransferase or inosine kinase.

Guanine is preferentially deaminated to xanthine in tobacco leaf protoplasts (Table 4). Conversion of guanine into GMP by guanine phosphoribosyltransferase (Table 5) and then into other compounds is one-quarter as much as deamination. Thus GMP is phosphorylated to GDP and GTP, and is finally incorporated into nucleic acids. The fact that the label was present in IMP and adenine nucleotides as well as in adenine and hypoxanthine (Table 4) may indicate that GMP reductase (EC 1.6.6.8) is active in protoplasts and that the sequence of reaction is: GMP → IMP → AMP. Thereafter AMP is phosphorylated to ADP and ATP or catabolized into adenine and hypoxanthine as described above. The relatively high incorporation of label of guanine into adenine nucleotides, as compared with animal tissue, indicates the importance of GMP reductase and GMP → IMP conversion in mesophyll tissue. Possibly the label observed in adenine and hypoxanthine compounds is derived from xanthine transformed into XMP by xanthine phosphoribosyltransferase. However, this problem calls for more detailed studies. The presence of small amounts of radioactive uric acid in protoplasts incubated with the individual precursors, as well as the enzymic findings, indicate that xanthine oxidoreductase is active in intact protoplasts. Xanthine oxidoreductase has so far been reported to occur in extracts of leaves of tobacco (Mendel & Müller, 1976), and *Pharbitis nil* (Nguyen, 1973) and in soyabean root nodules (Tajima & Yamamoto, 1975).

The present results enable us to propose the general scheme for metabolism of purine compounds in protoplasts of tobacco leaves (Scheme 1). This scheme is different from that generally accepted for animal tissues in the following aspects: (1) the nucleosides inosine, xanthosine and guanosine are not degraded to the corresponding bases, and these bases as well as adenine are not converted into the respective nucleosides, because of the lack of purine nucleoside phosphorylase; (2) adenosine is hydrolysed to adenine by adenosine nucleosidase (not found in animal tissues), but not deaminated to inosine because of the lack of adenosine amino-



Scheme 1. *Pattern of purine metabolism in mesophyll protoplasts of tobacco leaf*
Numbers represent the following enzymes: 1, adenine phosphoribosyltransferase; 2, hypoxanthine/guanine phosphoribosyltransferase; 3, adenosine nucleosidase; 4, adenine aminohydrolase; 5, inosine phosphotransferase and inosine kinase; 6, 5′-nucleosidase and phosphatases; 7, xanthine oxidoreductase; 8, guanine aminohydrolase; 9, adenylosuccinate synthetase and adenylosuccinate lyase; 10, IMP dehydrogenase; 11, GMP synthetase; 12, GMP reductase.

hydrolase (present in animal tissues); (3) adenine is deaminated to hypoxanthine by adenine aminohydrolase (absent from animal tissues); (4) adenosine cannot be converted into AMP, because of the lack of adenosine kinase (present in animal tissues), but inosine can be phosphorylated by inosine kinase or inosine phosphotransferase (enzymes not common in animal tissues).

The relationship between the purine metabolism and the intensity of RNA biosynthesis was investigated by using cultured protoplasts. After 24h of cultivation in the K_3 medium, mesophyll protoplasts enter into the stage preparatory to cell division. In such protoplasts the size and structure of nuclei are considerably changed and the synthesis of RNA, but not DNA (Zelcer & Galun, 1976), is enhanced. In our investigations we observed considerably higher purine incorporation into nucleic acids in these protoplasts. However, utilization of adenine and guanine, but not of hypoxanthine and adenosine (expressed as the sum of all radioactive compounds in Tables 1, 2, 3 and 4), increases, and only these two purines are incorporated more actively into nucleic acids, as compared with freshly obtained protoplasts. Also Suzuki & Takahashi (1977) have observed high incorporation of adenine and guanine and relatively low incorporation of hypoxanthine into RNA of excised tea (*Thea sinensis*) tips. Perhaps a low adenosine incorporation into nucleic acids during incubation of protoplasts with adenosine as compared with adenine results from a toxic effect of adenosine, which has been observed for animal cells (Harrap & Paine, 1977). In cultivated tobacco-leaf protoplasts not only incorporation of adenine and guanine into nucleic acids increases, but also both purines are deaminated faster to the corresponding oxypurines, and the rates of some catabolic and interconversion reactions are changed.

We thank Dr. M. M. Jeżewska of Institute of Biochemistry and Biophysics, Warsaw, Poland, for helpful discussions and suggestions in the preparation of the manuscript, Dr. J. F. Henderson of Cancer Research Unit, Edmonton, Alberta, Canada, and Dr. K. Kleczkowski of Institute of Biochemistry and Biophysics, Warsaw, Poland, for valuable discussion of the manuscript, and Miss H. Kadłubowska for her excellent technical assistance. This work was supported by the Polish Academy of Sciences within project 09.7.

References

Anderson, J. D. (1977) *Plant Physiol.* **59**, 610–614

Barankiewicz, J. & Henderson, J. F. (1977) *Biochem. Med.* **17**, 45–53

Barankiewicz, J. & Jeżewska, M. M. (1972) *Bull. Acad. Pol. Sci., Ser. Sci. Biol.* **20**, 1–4

Barnes, R. L. (1959) *Nature (London)* **184**, 1944

Brown, E. G. (1975) *Biochem. Soc. Trans.* **3**, 1199–1202

Clark, M. C. (1974) *J. Exp. Bot.* **25**, 309–319

Cocking, E. C. & Peberdy, J. F. (1974) *The Use of Protoplasts from Fungi and Higher Plants as a Genetic System: A Practical Handbook*, p. 67, Department of Botany, University of Nottingham, Nottingham

Farkas, W. R. & Singh, R. D. (1975) *Biochim. Biophys. Acta* **377**, 166–173

Guranowski, A. & Schneider, Z. (1977) *Biochim. Biophys. Acta* **482**, 145–158

Harrap, K. R. & Paine, R. M (1977) *Adv. Enzyme Regul.* **15**, 169–193

Henderson, J. F. & Patterson, A. R. P. (1973) *Nucleotide Metabolism*, pp. 97–199, Academic Press, New York and London

Henderson, J. F., Fraser, J. H. & McCoy, E. E. (1974) *Clin. Biochem.* **7**, 339–358

Kao, K. N., Constabel, F., Michayluk M. R. & Gamborg, O. L. (1974) *Planta* **120**, 215–227

Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **193**, 265–275

Mendel, R. R. & Müller, A. J. (1976) *Biochem. Physiol. Pflanz.* **170**, 538–541

Nguyen, J. (1973) *C.R. Hebd. Seances Acad. Sci. Ser. D* **273**, 3025–3027

Nicholls, P. P. & Murray, A. W. (1968) *Plant Physiol.* **43**, 645–648

Power, I. B., Frearson, E. M. & Cocking, E. C. (1971) *Biochem. J.* **123**, 29P–30P

Price, C. E. & Murray, A. W. (1969) *Biochem. J.* **115**, 129–133

Schlee, D. & Reinbothe, H. (1965) *Phytochemistry* **4**, 311–315

Snyder, F. F. & Henderson, J. F. (1973) *J. Cell. Physiol.* **82**, 349–361

Suzuki, T. & Takahashi, E. (1977) *Drug Metab. Rev.* **6**, 213–242

Tajima, S. & Yamamoto, Y. Y. (1975) *Plant Cell Physiol.* **16**, 271–282

Turner, D. H. & Turner, J. F. (1961) *Biochem. J.* **79**, 143–147

Zelcer, A. & Galun, E. (1976) *Plant Sci. Lett.* **7**, 331–336